# PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PO Box 2219
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## PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing *day/month/year* | - 6 FEB 2004 |
| Applicant's or agent's file reference<br>2601959/VPA/sjp | **IMPORTANT NOTIFICATION** |

| International Application No. | International Filing Date | Priority Date |
|---|---|---|
| **PCT/AU2002/001768** | 30 December 2002 | 28 December 2001 |

Applicant

DELTA BIOTECHNOLOGY LIMITED et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

| Name and mailing address of the IPEA/AU | Authorized officer |
|---|---|
| AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | JAMIE TURNER<br>Telephone No. (02) 6283 2071 |

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>2601959/VPA/sjp | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
|---|---|---|
| International Application No.<br>**PCT/AU2002/001768** | International Filing Date *(day/month/year)*<br>30 December 2002 | Priority Date *(day/month/year)*<br>28 December 2001 |
| International Patent Classification (IPC) or national classification and IPC<br>**Int. Cl.** [7] C12N 1/20 | | |
| Applicant<br>DELTA BIOTECHNOLOGY LIMITED et al | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **3** sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

| Date of submission of the demand<br>1 July 2003 | Date of completion of the report<br>3 February 2004 |
|---|---|
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**JAMIE TURNER**<br>Telephone No. (02) 6283 2071 |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2002/001768

## I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-74,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the claims, pages **75,** as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **76-78,** received on **28 January 2004** with the letter of **28 January 2004**

☒ the drawings, pages **1/8 - 8/8,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1-13,** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims **1-39** | **YES** |
| | Claims | **NO** |
| Inventive step (IS) | Claims **1-39** | **YES** |
| | Claims | **NO** |
| Industrial applicability (IA) | Claims **1-39** | **YES** |
| | Claims | **NO** |

2. Citations and explanations (Rule 70.7)

The following documents, first raised in the corresponding International Search Report, are referred to as follows:

D1 - FEMS Microbiol. Lett., Vol. 221, 2003, pages 7-16
D2 - Infect. Immun., Vol. 58, No. 3, March 1990, pages 732-9
D3 - EP 0 400 958
D4 - EP 0 574 466
D5 - EP 1 108 790
D6 - AU 709385

The claims of the present international application relate to a modified *Bordetella* strain having a loss of function in the endogenous *aroQ* gene and a lower capacity to propagate in a mammalian host but remaining viable in the host for a time sufficient to induce an immune response against pathogenic *Bordetella* strain. It also relates to nucleic acid constructs for disrupting an *aroQ* gene in a *Bordetella* cell (comprising a replacement portion, a first homology region upstream of the replacement portion and a second homology region downstream of the replacement region) and to isolated polynucleotides (*Bordetella aroQ* gene and coding sequence thereof) and 3-dehydroquinase encoded by the *aroQ* gene.

The most relevant prior art document, D1, relates to the construction of a *Bordetella pertussis* strain containing a mutated *aroA* gene. It further teaches that the strain was used to induce an immune response in mice. The document does not disclose *aroQ* mutants. Hence, D1 does not detract from the novelty or inventive step of the claims.

Documents D5 and D6 each disclose sequences which share some identity with SEQ ID NOs: 1-3. However, the claims to the sequences per se are limited to either a specific length (50 nucleotides) or to a specific sequence identity (70%). Hence, D5 and D6 do not adversely affect the novelty or inventive step of claims of the present application.

9. The genetically modified strain of claim 5, comprising an exogenous nucleic acid sequence in its genome, or on an extrachromosomal element, which is capable of abolishing or otherwise reducing the expression of *aroQ* or the level and/or functional activity of the 3-dehydroquinase encoded by *aroQ*, wherein the nucleic acid sequence comprises a ribozyme-encoding polynucleotide that is operably linked to a transcriptional control element, wherein the ribozyme specifically binds to or otherwise interacts with a transcript of the *aroQ* gene.

10. The genetically modified strain of claim 1, further having a partial or complete loss of function in at least one other endogenous gene selected from a *pur* gene, another *aro* gene, a pertussis toxin gene, or any other gene which contributes to survival in the host and/or to bacterial virulence, or a combination thereof.

11. The genetically modified strain of claim 1, wherein the *pur* gene is selected from *purA*, *purE* or *purH*.

12. The genetically modified strain of claim 1, wherein the *aro* gene is selected from *aroA*, *aroB*, *aroC* or *aroE*.

13. The genetically modified *Bordetella* strain of claim 1, comprising at least one exogenous gene which is capable of expressing an antigen that is heterologous or foreign to the *Bordetella* strain.

14. The genetically modified *Bordetella* strain of claim 13, wherein the heterologous or foreign antigen is derived from a pathogen that is unrelated to the *Bordetella* strain.

15. The genetically modified *Bordetella* strain of claim 13, wherein the heterologous or foreign antigen is derived from a pathogen that infects by the mucosal route.

16. An isolated polynucleotide comprising a nucleotide sequence that corresponds or is complementary to at least a portion of the sequence set forth in SEQ ID NO: 1 or 3, which portion is at least 50 nucleotides in length.

17. The polynucleotide of claim 16, wherein the nucleotide sequence has at least 70% sequence identity to at least a portion of the sequence set forth in SEQ ID NO: 1 or 3.

18. The polynucleotide of claim 16, wherein the nucleotide sequence is capable of hybridising to at least a portion of the sequence set forth in SEQ ID NO: 1 or 3 under at least medium stringency conditions.

19. The polynucleotide of claim 16, wherein the portion is a biologically active fragment of the sequence set forth in SEQ ID NO: 1 or 3.

20. An isolated polypeptide comprising an amino acid sequence that has at least 70% sequence identity to at least a portion of the sequence set forth in SEQ ID NO: 2.

21. The polypeptide of claim 20, wherein the portion is at least 6 amino acids in length.

22. The polypeptide of claim 20, wherein the portion is a biologically active fragment of the sequence set forth in SEQ ID NO: 2.

23. A nucleic acid construct for disrupting an *aroQ* gene in a *Bordetella* cell, comprising: a) a non-homologous replacement portion; b) a first homology region located upstream of the non-homologous replacement portion, the first homology region having a nucleotide sequence with substantial identity to a first *aroQ* gene sequence; and c) a second homology region located downstream of the non-homologous replacement portion, the second homology region having a nucleotide sequence with substantial identity to a second *aroQ* gene sequence, the second *aroQ* gene sequence having a location downstream of the first *aroQ* gene sequence in a naturally occurring endogenous *aroQ* gene of the *Bordetella* cell.

24. The construct of claim 23, wherein the *aroQ* gene comprises the sequence set forth in SEQ ID NO: 1 or 3 or a variant or derivative thereof.

25. A vector comprising a nucleotide sequence that corresponds or is complementary to at least a portion of the sequence set forth in SEQ ID NO: 1 or 3, which portion if at least 50 nucleotides in length.

26. The vector of claim 25, wherein the vector is a DNA targeting vector.

27. A host cell containing the construct of claim 23 or the vector of claim 25.

28. An antigen-binding molecule that is specifically interactive with the polypeptide of claim 20.

29. A method for producing a genetically modified *Bordetella* strain, comprising introducing the nucleic acid construct of claim 23 into a *Bordetella* cell under conditions such that the nucleic acid construct is homologously recombined into the *aroQ* gene in the genome of that cell to produce a genetically modified *Bordetella* cell containing a disrupted *aroQ* gene.

30. The method of claim 29, wherein the genetically modified *Bordetella* cell containing the homologously recombined nucleic acid construct is further characterised by expressing reduced or undetectable levels of *aroQ*.

31. The method of claim 29, wherein the genetically modified *Bordetella* cell lacks the ability to produce a functional 3-dehydroquinase encoded by said *aroQ* gene.

32. A composition, comprising the genetically modified *Bordetella* strain of claim 1, together with a pharmaceutically acceptable carrier.

33. The composition of claim 32, further comprising an adjuvant.

34. A composition of matter comprising dendritic cells which have been exposed to the genetically modified *Bordetella* strain of claim 1 for a time and under conditions sufficient to express a processed or modified antigen derived from the *Bordetella* strain for presentation to, and modulation of, T cells.

35. The composition of matter of claim 34, which is in the form of an *in vitro* cell culture.

36. A method for modulating an immune response, comprising administering to a patient in need of such treatment an effective amount of the genetically modified *Bordetella* strain of claim 1, or the composition of claim 32 or the composition of matter of claim 34.

37. A method for the treatment and/or prophylaxis of whooping cough or related condition, comprising administering to a patient in need of such treatment an effective amount of the genetically modified *Bordetella* strain of claim 1, or the composition of claim 32 or the composition of matter of claim 34.

38. Use of the genetically modified *Bordetella* strain of claim 1 in the study, and modulation of an immune response.

39. The use of claim 38, wherein the immune response is against a pathogenic strain of *Bordetella* or related organism.